Friction analysis of kinetic schemes

Lolkema, Juke S.

Published in:
Biochimica et biophysica acta-Protein structure and molecular enzymology

DOI:
10.1016/0167-4838(95)00112-8

Publication date:
1995

ELSEVIER

Biochimica et Biophysica Acta 1252 (1995) 284–294

BBA Biochimica et Biophysica Acta

Friction analysis of kinetic schemes: the friction coefficient

Juke S. Lolkema *

Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Received 16 February 1995; accepted 13 May 1995

Abstract

Friction analysis is proposed as the application of general control analysis to single enzymes to describe the control of elementary kinetic steps on the overall catalytic rate. For each transition, a friction coefficient is defined that measures the sensitivity of the turnover rate to the free energy of the transition state complex of the transition. The latter is captured in a single property of the transition, termed friction, as the geometrical mean of the inverse of the forward and backward rate constants. By definition, the friction coefficient measures the relative change in the turnover rate in response to a small change in the friction. The friction coefficient is the sum of the flux control coefficients of the forward and backward rate constants from general control theory and measures the extent to which an elementary step is rate determining. Two basic rules apply to the friction coefficients: (i) the summation theorem states that summation of the friction coefficients over all the steps in a scheme results in a value of 1, and (ii) the group rule states that grouping of rate constants of similar transitions results in a friction coefficient for the group that is the sum of the friction coefficients of the individual steps in the group. The friction coefficients are derived for a number a kinetic schemes taking the rate equations as the starting point and both rules are demonstrated. In fully coupled systems the friction coefficients of individual steps lie between 0 and 1. In partially uncoupled systems the summation theorem applies to all the rates in the system, however, the summation of subsets of friction coefficients may exceed the value of one, implying negative values for other steps in the scheme. The values of individual friction coefficients lie between -1 and 1. The friction coefficient is redefined in a numerical treatment of the steady state of more complex enzymatic schemes.

Keywords: Friction analysis; Friction coefficient; Enzyme kinetics; Control theory

1. Introduction

The control theory of metabolic pathways describes to what extent the enzymes that catalyse the individual steps in the pathway determine the flux through the pathway by virtue of the control coefficients (for recent reviews see [1–3]). The control coefficient of an enzyme measures the relative change in the overall flux in response to a small change in the enzyme concentration. Experimentally, the control coefficients may be determined by changing the enzyme concentration from expression-controlled recombinant plasmids or by inhibitor titration (e.g., [4,5]). Knowledge of the control coefficients provides, for one thing, information about the 'key enzymes' in a metabolic pathway.

A kinetic scheme that describes the catalytic mechanism of a single enzyme bares a lot of resemblance to a metabolic pathway. The metabolic intermediates connected by the enzyme catalysed conversions are replaced by the states of the enzyme connected by the transitions between the states. The flux through the pathway is analogues to the flux through the enzyme, i.e., the turnover rate. A control theory for enzyme catalysis seems to be appropriate in dealing with the concept of 'rate-limiting steps' as was recognized in recent publications [6,7]. However, even though the mathematical description of the systems is the same, important differences between a metabolic pathway and a kinetic scheme make it better not to use the same terminology in both cases. The most important difference is that the parameters controlling the flux in a metabolic pathway and an enzymatic scheme are different, enzyme concentration and activation energy, respectively. Furthermore, while the system variables in the single enzyme case, i.e., the rate constants, are related via the principle of microscopic reversibility, the enzyme concentrations in the metabolic pathway are independent. Consequently, control analysis of single enzymes is a special case of the general

* E-mail: j.s.lolkema@biol.rug.nl. Fax: +31 50 632154.

SSDI 0167-4838(95)00112-3

control theory. Here, 'frictional analysis' is proposed as the flux control theory for enzymatic schemes and the friction coefficient as the analogue of the flux control coefficient. Then, the friction coefficient of an elementary step in a kinetic scheme determines the extent to which that step controls the rate under specified conditions [8]. Classical kinetic analysis results in the rate equation pertinent to a kinetic scheme. Frictional analysis is an extension that analyses the control of the individual steps over the overall rate.

The justification to set up the frictional analysis is twofold. One, numerical techniques allow the evaluation of the friction coefficients of each step in kinetic schemes of any complexity without knowing the analytical function for the overall rate equation. Especially with complex kinetic schemes, the friction coefficient is an important tool in understanding the kinetic behaviour of the enzyme. The tool was successfully used in the analysis of the complex phosphorylation kinetics of the mannitol transporter of the *Escherichia coli* phospho*enol*pyruvate-dependent phosphotransferase system [8]. Two, the activation energies in a kinetic scheme can be experimentally modified by the widely used technique of site-directed mutagenesis. Before this technique became common place, the kinetic isotope effect was the only experimental handle, with a very limited scope, to effect activation energies [9]. The ability to make site-directed mutant enzymes does not imply that the activation energy of selected transitions in the scheme can be changed. More likely, multiple steps are affected both with respect to activation energies and the free energies of the states. Nevertheless, frictional analysis will help to pin-point the mutation in the kinetic scheme by predicting, based upon the wild-type kinetic scheme, under which conditions changes in elementary steps will or will not show up in the experimental kinetics. Especially in energy transducing (multi cycle) enzymes, frictional analysis will help to explain how mutations that, for instance, uncouple two substrate fluxes or induce leaks, e.g., [10–15] or wild-type properties like electrogenic steps work out in the experimental kinetic behaviour of the enzyme.

Ray, 1983 [9] was the first to treat the concept of 'rate-limiting steps' in terms of extent of control of the elementary steps on the overall rate when dealing with the kinetic isotope effect. Lolkema, 1993 [8] used a similar approach in the analysis of the mannitol transporter of the bacterial phosphotransferase system. Brown and Cooper, 1993 [6] and a little later, but much more extensively, Kholodenko and Westerhoff, 1994 [7] were the first to give an explicit treatment in terms of general control theory. The main focus in the latter paper was on the summation and connectivity theorems using matrix notations which may not be very appealing to enzyme kineticists. The implications of the dependence between the rate constants in a kinetic scheme and the actual expressions of the flux control coefficients in the rate constants and the ligand concentrations have gained much less attention. In this contribution the friction coefficient will be defined within the transition state theory. The rules to which the friction coefficients in a scheme are subject will be discussed and analytically demonstrated taking the rate equations as the starting point. This results in many expression for the friction coefficients. In addition, the implementation of the friction coefficient in a numerical analysis of enzyme kinetics [8] will be discussed.

2. Friction and the friction coefficient

The steady state rate equation describes the catalytic rate as a function of the ligand concentrations and the rate constants. The latter are properties of the elementary kinetic steps. General control theory describes the control of any system parameter, e.g., a rate constant, on the rate by measuring the relative change in the rate in response to a small change in the system parameter [6]. In a kinetic scheme the rate constants are related via the principle of microscopic reversibility [16] and changing a single rate constant results in a physical irreality. Moreover, control theory of single enzymes is meant to describe the control exerted by an elementary 'step' rather than a rate constant. Therefore, the control of an elementary step has been defined by changing both the forward and backward rate constant at the same time by the same relative amount [7–9] which meets both objections. The meaning of changing both rate constants pertinent to a transition is demonstrated in Fig. 1 that shows the free energy profile of the transition between enzyme states E_i and E_j isolated from the rest of the kinetic scheme. The free energies of states E_i and E_j are G_i and G_j, respectively. The free energy at the top of the energy barrier between the two states, i.e., the free energy of the transition state, is $G^{\#}$. The forward (arbitrarily chosen as the i to j direction) rate constant k^+ and the backward rate constant k^- are associated with the

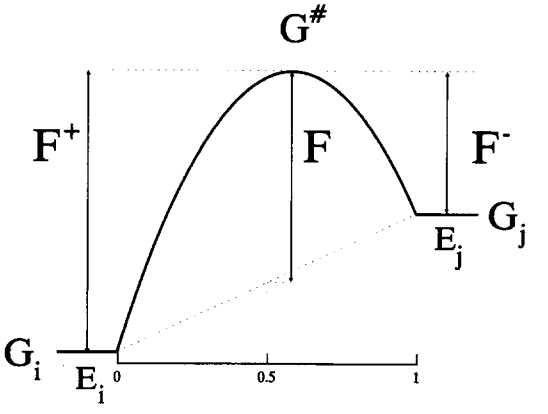


Fig. 1. Friction in a transition between two states of an enzyme. The free energy profile is described as an inverted parabola according to Marcus' formalism [19]. See the text for explanation.

free energy differences $G^{\#} - G_i$ and $G^{\#} - G_j$, respectively,

$$k^+ = k^0 \exp\frac{-(G^{\#} - G_i)}{RT} \quad k^- = k^0 \exp\frac{-(G^{\#} - G_j)}{RT} \tag{1}$$

A relative change in both k^+ and k^- results from a change in the free energy of transition state $G^{\#}$,

$$\frac{dk^+}{k^+} = \frac{dk^-}{k^-} = -\frac{dG^{\#}}{RT} \tag{2}$$

The property of the elementary step that defines the control of the step on the overall rate is the free energy level of the transition state which can be captured in a single system parameter, friction F, as follows. Intuitively, rate control increases with the free energy of the transition state and, therefore, is related to the reciprocal of the rate constants for which frictions $F^+ = 1/k^+$ and $F = 1/k^-$ may be introduced. By definition, the friction F of the transition is the geometrical mean of the two directional frictions F^+ and F^- which is related to the free energy difference between $G^{\#}$ and $G_i + 1/2(G_j - G_i)$ (see Fig. 1),

$$F = F^0 \exp\frac{G^{\#} - \frac{1}{2}G_i - \frac{1}{2}G_j}{RT} \tag{3}$$

in which F^0 equals $1/k^0$. Some algebra reveals Eq. (4) which relates the friction of the transition between states E_i and E_j to the frictions and rate constants in the forward and backward direction.

$$F = \sqrt{F^+F^-} = \sqrt{\frac{1}{k^+k^-}} \tag{4}$$

For the present purpose, changes in the friction should reflect changes in the free energy of the transition state and not of the two states E_i and E_j. This is achieved by expressing one of the rate constants in the equilibrium constant K of the transition,

$$F = \frac{1}{k^+}\sqrt{K} = \frac{1}{k^-}\sqrt{\frac{1}{K}} \tag{5}$$

The condition that K is treated as a constant when F changes implicitly means that both rate constants change at the same time. Friction F can now be used as the property of an elementary step by which the control on the overall rate can be established. The extent of rate determination by a particular step in a kinetic scheme is determined by the sensitivity of the turnover rate to an infinite small change in the friction of that step. A friction coefficient f_i may be defined for each step i that measures the relative change in the turnover rate (v) in response to a relative change in the friction F_i

$$f_i = -\frac{\delta v/v}{\delta F_i/F_i} \tag{6}$$

The minus sign is included in this definition since intuitively, but not necessarily, an increase in the friction will result in a decrease of the turnover rate. The derivative of v to F is a partial derivative indicating that the frictions of all other steps are kept constant. The formal definition of the friction coefficient in Eq. (6) is not a very convenient one for further manipulation since the rate equations will be in terms of rate constants rather than frictions. A more operational definition follows by combining Eqs. (6) and (5)

$$f_i = \frac{k_i^+}{v}\left(\frac{\delta v(k_i^+)}{\delta k_i^+}\right)_{K_i = \text{const.}} \tag{7}$$

in which the forward rate constant was arbitrarily chosen as the variable. Eq. (7) is equivalent to the definition of the flux control coefficients given at the beginning of this paragraph. The friction coefficient is the sum of the flux control coefficients of k^+ and k^- from general control theory. Brown and Copper [6] have argued that this grouping is arbitrary and therefore restrictive. Here, it is argued that the sum of the control coefficients of the forward and backward rate constants, in fact, measures what we would like to know: the extent to which a elementary step in a kinetic mechanism is rate determining. The summation of the two control coefficients is a logical consequence of the definition of rate control in terms of the free energy of the transition state.

3. The summation theorem

The friction coefficients of the transitions in a kinetic scheme behave according to certain rules, the most prominent one being that the sum of the friction coefficients over all transitions i equals one

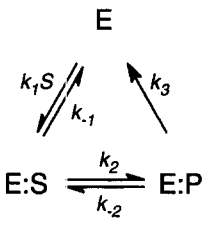
$$\sum_i f_i = 1 \tag{8}$$

Mathematically, the summation theorem is general to functions that are homogenous and first order in the variables. The theorem will be demonstrated for a number of cases for which the analytical rate equations are available. In doing so, analytical expressions for the friction coefficients will be derived for these schemes and the limits for the numerical values of the friction coefficients will be revealed.

3.1. One-substrate reactions

Irreversible

Scheme I shows a simple catalytic cycle of an enzyme that converts substrate S into product P under conditions where the product concentration is kept zero.



Scheme I

The rate equation in terms of the rate constants and substrate concentration S pertinent to this scheme is

$$v = \frac{k_1 k_2 k_3 S}{k_{-1}k_3 + k_{-1}k_{-2} + k_2 k_3 + k_1 k_2 S + k_1 k_3 S + k_1 k_{-2} S} \quad (9)$$

In order to derive friction coefficients f_i (Eq. 7), the forward rate constant is arbitrarily selected as the variable and the backward rate constant is expressed in the variable via the expression for equilibrium constant K_i. This results in

$$v = \frac{k_1 k_2 k_3 S}{\frac{k_1 k_3}{K_1} + \frac{k_1 k_2}{K_1 K_2} + k_2 k_3 + k_1 k_2 S + k_1 k_3 S + \frac{k_1 k_2 S}{K_2}} = \frac{k_1 k_2 k_3 S}{\Delta} \quad (10)$$

For each rate constant k_i this can be written as

$$v(k_i) = \frac{A_i k_i}{B_i + C_i k_i} \quad (11)$$

with i ranging from 1 to 3. A_i, B_i and C_i are constants. Variable selection simply follows from reorganization of the numerator and denominator from Eq. (10). Therefore,

$$A_1 k_1 = A_2 k_2 = A_3 k_3 = A_i k_i \quad (12)$$

and

$$B_1 + C_1 k_1 = B_2 + C_2 k_2 = B_3 + C_3 k_3 = B_i + C_i k_i \quad (13)$$

In Table 1 is indicated to which B_i-term the respective terms in the denominator summation contribute. Each term contributes to a single B_i-term which leads to an additional relation for B_i

$$\sum_{i=1}^{3} B_i = B_i + C_i k_i \quad (14)$$

The partial derivative of rate Eq. (11) to k_i equals

$$\frac{\delta v(k_i)}{\delta k_i} = \frac{A_i}{B_i + C_i k_i} - \frac{A_i C_i k_i}{(B_i + C_i k_i)^2} \quad (15)$$

and, consequently, it follows for the friction coefficient from Eq. (7)

$$f_i = 1 - \frac{C_i k_i}{B_i + C_i k_i} = \frac{B_i}{B_i + C_i k_i} \quad (16)$$

Table 1
Analysis of the denominators Δ in rate Eqs. (10), (18) and (19)

One-substrate irreversible		One-substrate reversible		Two-substrates consecutive	
Δ	B_i	Δ	B_i	Δ	B_i
k_1k_3/K_1	B_2	k_1k_2/K_1K_2	B_3	$k_1k_2k_3AB$	B_4
k_1k_2/K_1K_2	B_3	k_1k_3/K_1	B_2	$k_1k_3k_4AB$	B_2
k_2k_3	B_1	k_2k_3	B_1	$k_1k_2k_3A/K_3$	B_4
k_1k_2S	B_3	k_1k_2S	B_3	$k_1k_2k_4A$	B_3
k_1k_3S	B_2	k_1k_2S/K_2	B_3	$k_1k_3k_4B/K_1$	B_2
k_1k_2S/K_2	B_3	k_1k_3S	B_2	$k_2k_3k_4B$	B_1
		k_1k_3P/K_1K_3	B_2		
		k_2k_3P/K_3	B_1		
		k_2k_3P/K_2K_3	B_1		

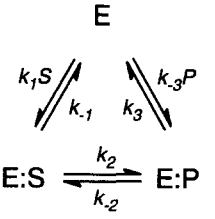
Denominators Δ are the sum of the terms in the first column. The second column indicates for each term to which B_i-term it contributes. For all three cases each term in Δ contributes to a single B_i-term and, therefore, $\Sigma B_i = \Delta$

Since all the terms in B_i and $C_i k_i$ are positive it follows that the friction coefficients are numbers between 0 and 1. Together with Eq. (14) it follows that the sum of the friction coefficients of the three transitions equals 1:

$$\sum_{i=1}^{3} f_i = \frac{\sum_{i=1}^{3} B_i}{B_i + C_i k_i} = 1 \quad (17)$$

Reversible

Any rate equation that can be written in the form of Eq. (11) will result in the expression of Eq. (16) for friction coefficients f_i. The summation over the friction coefficients will be 1 when condition (14) is fulfilled. Kinetic scheme II shows the reversible case of the enzyme depicted in kinetic scheme I.



Scheme II

The rate equation becomes more complicated and, after eliminating the backward rate constant for each transition, takes the form

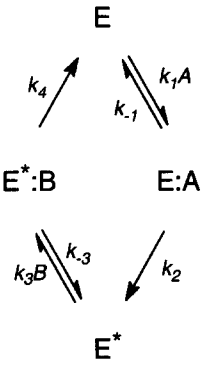
$$v = \frac{(S - P/K_1K_2K_3)k_1k_2k_3}{\Delta} \quad (18)$$

in which Δ is the sum of the 9 terms listed in Table 1. Clearly, Eq. (18) may be written in the form of Eq. (11) and condition (14) is fulfilled, since all terms in the denominator contribute to a single B_i-term (see Table 1). Consequently, the friction coefficients add up to unity.

3.2. Two-substrate reactions

Consecutive mechanism

In the 'ping-pong' type reaction depicted in Scheme III the enzyme first reacts with substrate A to form an enzyme state E^* which subsequently reacts with substrate B to regenerate the original state of the enzyme. Such a scheme is an additional example that results in a rate equation of the form of Eq. (11) for which condition (14) holds.



Scheme III

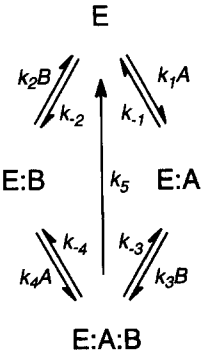
The steady state solution is

$$v = \frac{k_1 k_2 k_3 k_4 \mathrm{AB}}{\Delta} \tag{19}$$

The terms that make up denominator Δ together with the distribution over the B_i-terms are given in Table 1.

Parallel pathways

Kinetic scheme IV shows the catalytic cycle of an enzyme that forms a ternary complex with two substrates, A and B, from which product P is formed. The order of binding of substrates A and B is random.



Scheme IV

The steady state solution for the rate equation may be found by the method of King and Altman [17] and this particular case is described in the textbook of Dixon and Webb [18]. In contrast to the schemes described above, the numerator of the rate equation contains terms that do no contain all the five rate constants pertinent to the five transitions in the scheme. Consequently, the rate equation takes the form

$$v(k_i) = \frac{A_i k_i + D_i}{B_i + C_i k_i} = \frac{\eta}{\Delta} \tag{20}$$

Nominator η and denominator Δ are the sum of the terms listed in the first column of Table 2. Using Eq. (7) it may be derived that the expression for the friction coefficients in the scheme is

$$f_i = \frac{A_i k_i}{A_i k_i + D_i} - \frac{C_i k_i}{B_i + C_i k_i} \tag{21}$$

Table 2
Analysis of the numerator η (top) and denominator Δ (bottom) of rate Eq. (20)

η	A_1k_1	A_2k_2	A_3k_3	A_4k_4	A_5k_5
$k_1k_2k_3k_5\ AB/K_2$	*	*	*		*
$k_1k_2k_4k_5\ AB/K_1$	*	*		*	*
$k_1k_3k_4k_5\ A^2B$	*		*	*	*
$k_2k_3k_4k_5\ AB^2$		*	*	*	*
Δ	C_1k_1	C_2k_2	C_3k_3	C_4k_4	C_5k_5
$k_1k_2k_3\ (1/K_1K_2K_3 + A/K_2K_3 + B/K_3 + AB/K_2)$	*	*	*		
$k_1k_2k_4\ (1/K_1K_2K_4 + A/K_2K_4 + B/K_1K_4 + AB/K_1)$	*	*		*	
$k_1k_2k_5\ (1/K_1K_2K_5 + A/K_2 + B/K_1)$	*	*			*
$k_1k_3k_4\ (A/K_1K_3 + AB/K_4 + A^2/K_3 + A^2B)$	*		*	*	
$k_1k_4k_5\ (A/K_1 + A^2)$	*			*	*
$k_2k_3k_4\ (B/K_2K_4 + AB/K_3 + B^2/K_4 + AB^2)$		*	*	*	
$k_2k_3k_5\ (B/K_2 + B^2)$		*	*		*
$k_3k_4k_5\ AB$			*	*	*

Numerator η and denominator Δ are the sum of the terms in the first column. A_ik_i (top) and C_ik_i (bottom) equal the sum of the terms indicated by the asterisk (*) in the corresponding column. The remaining terms equal the D_i (top) and B_i (bottom) terms. Since each term in η contributes four times to the respective A_ik_i-terms and one time to the D_i-terms it follows that $\Sigma A_ik_i = 4\eta$ and $\Sigma D_i = \eta$. Similarly, each term in the denominator is represented three times in the C_ik_i-terms and two times in the B_i-terms and, therefore, $\Sigma C_ik_i = 3\Delta$ and $\Sigma B_i = 2\Delta$

and, consequently, it follows for the summation over the friction coefficients

$$\sum_{i=1}^{5} f_i = \frac{\sum_{i=1}^{5} A_i k_i}{A_i k_i + D_i} - \frac{\sum_{i=1}^{5} C_i k_i}{B_i + C_i k_i} \tag{22}$$

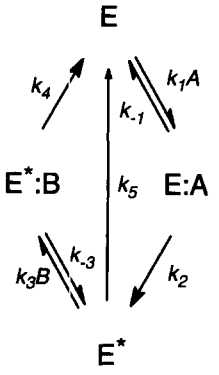
The contribution of the terms in the sum of numerator η and denominator Δ of rate Eq. (20) to the $A_i k_i$-terms and the $C_i k_i$-terms, respectively, are analysed in Table 2. It follows that

$$\frac{\sum_{i=1}^{5} A_i k_i}{A_i k_i + D_i} = 4 \quad \frac{\sum_{i=1}^{5} C_i k_i}{B_i + C_i k_i} = 3 \tag{23}$$

Substitution into Eq. (22) shows that the summation over the friction coefficients of the five transitions adds up to 1.

Partially coupled systems

In the above two cases the enzyme catalyses the disappearance of substrates A and B at exactly the same rate. The two fluxes are fully coupled. Scheme V shows once again the 'ping-pong' mechanism depicted in Scheme III, but with an additional transition between states E* and E.



Scheme V

The state created by reaction of the enzyme with the first substrate relaxes back with a significant rate constant to the original state, thereby introducing a slip in the enzyme. An example of such an enzyme would be a kinase that phosphorylates a sugar substrate (B) from ATP (A) via an phosphorylated enzyme intermediate (E*). The enzyme catalyses three different fluxes, the rate of disappearance of B, which in most cases will be the rate of product formation (ν_B), the rate of disappearance of A (ν_A) and the rate of slippage (ν_{slip}). In the example, these would be the rate of sugar phosphorylation, the rate of ATP hydrolysis and the rate of hydrolysis of the phospho-enzyme intermediate, respectively. The analytical expressions for the three rates are

$$\upsilon_B = \frac{k_1 k_2 k_3 k_4 AB}{\Delta} = \frac{\eta_B}{\Delta} \tag{24}$$

$$\upsilon_A = \frac{\eta_B + k_1 k_2 k_5 (k_3/K_3 + k_4)}{\Delta} = \frac{\eta_A}{\Delta} \tag{25}$$

$$\upsilon_{slip} = \frac{\eta_A - \eta_B}{\Delta} = \frac{\eta_{slip}}{\Delta} \tag{26}$$

See Table 3 for the terms in denominator Δ. Denominator Δ in Eqs. (24), (25) and (26) is different from the denominator in Eq. (19) that gives the rate equation for the enzyme without the slip (see Table 3 and 1, respectively). Clearly, the sum of the rates of product formation and slipping equals the rate of disappearance of substrate A. Since the friction coefficients in the kinetic scheme measure the effect of a small change in the friction of the transitions on the rate, three sets of friction coefficients can be defined, f_i^B, f_i^A and f_i^{slip}. The rate of product formation ν_B expressed in rate constants k_i takes the form of Eq. (11) for values of i from 1 through 4, and, consequently

$$f_i^B = \frac{B_i}{B_i + C_i k_i} \quad i = 1,2,3,4 \tag{27}$$

However, analysis of the B_i-terms in denominator Δ (Table 3) reveals that condition (14) is not fulfilled. Instead, it follows

$$\sum_{i=1}^{4} B_i = B_i + C_i k_i + C_5 k_5 \tag{28}$$

and, consequently, for the sum over the friction coefficients of transitions 1 through 4

$$\sum_{i=1}^{4} f_i^B = 1 + \frac{C_5 k_5}{B_5 + C_5 k_5} \tag{29}$$

With respect to the rate of product formation, introduction of the slip in Scheme III results in a numerical value for the sum over the friction coefficients of transitions 1 through 4 that lies in between 1 and 2. Reorganization of rate Eq. (24) shows that

$$\upsilon_B(k_5) = \frac{D_5}{B_5 + C_5 k_5} \tag{30}$$

With the help of Eq. (7) it follows for the friction coefficient of the slipping step

$$f_5^B = -\frac{C_5 k_5}{B_5 + C_5 k_5} \tag{31}$$

The friction coefficient of the slip with respect to the rate of product formation is a negative number between 0 and -1. The negative sign indicates that an increase in the friction in the slip results in an increase of the rate of

Table 3
Analysis of numerators η_A and η_{slip} (top) and denominator Δ (bottom) in rate Eqs. (24), (25) and (26)

η_A	A_3k_3	A_4k_4	A_5k_5	η_{slip}	A_3k_3	A_5k_5
$k_1k_2k_3k_4\ AB$	*	*		$k_1k_2k_4k_5A$		*
$k_1k_2k_4k_5\ A$		*	*	$k_1k_2k_3k_5\ A/K_3$	*	
$k_1k_2k_3k_5\ A/K_3$	*		*			

Δ	B_1	B_2	B_3	B_4	B_5	C_3k_3	C_4k_4	C_5k_5
$k_1k_2k_3\ (AB + A/K_3)$				*	*	*		
$k_1k_3k_4\ (AB + B/K_1)$			*		*	*	*	
$k_1k_2k_4\ A$			*		*		*	
$k_2k_3k_4\ B$	*				*	*	*	
$k_1k_4k_5\ (A + 1/K_1)$		*	*				*	*
$k_1k_3k_5\ (A/K_3 + 1/K_1K_3)$		*		*		*		*
$k_2k_4k_5$	*		*				*	*
$k_2k_3k_5/K_3$	*			*		*		*

Numerator η_A and denominator Δ are the sum of the terms in the first column. A_ik_i (top) and B_i and C_ik_i (bottom) equal the sum of the terms indicated by the asterisk (*) in the corresponding column. Each term in η_A contributes twice to the A_ik_i-terms with $i = 3,4,5$. Therefore, $A_3k_3 + A_4k_4 + A_5k_5 = 2\eta_A$. Similar, each term in η_{slip} is represented once in A_3k_3 and A_4k_4, so $A_3k_3 + A_4k_4 = \eta_{slip}$. The top four terms in Δ are represented oncein the B_i-terms for $i = 1$ through 4. The bottom four terms that make up C_5k_5, are represented twice in the same B_i-terms. Therefore, for $i = 1$–4, $\Sigma B_i = \Delta + C_5k_5$. The sum of B_i and B_2 equals Δ minus the first and the third term. Similarly, the sum of C_ik_i for $i = 3$ through 5 equals 2Δ minus the same two terms. Therefore, $B_1 + B_2 - (C_3k_3 + C_4k_4 + C_5k_5) = -\Delta$.

product formation, which intuitively is correct. Eq. (31) together with Eq. (29) shows that the sum over the friction coefficients of all the transitions in the scheme equals 1.

Variable selection reveals two different expressions for the rate of disappearance of substrate A depending on the value of i. For $i = 1,2$ and for $i = 3,4,5$ rate Eq. (25) takes the form of Eqs. (11) and (20), respectively, and consequently

$$f_i^A = \frac{B_i}{B_i + C_ik_i} \quad i = 1,2 \tag{32}$$

$$f_i^A = \frac{A_ik_i}{A_ik_i + D_i} - \frac{C_ik_i}{B_i + C_ik_i} \quad i = 3,4,5 \tag{33}$$

It follows for the sum of the friction coefficients

$$\sum_{i=1}^{5} f_i^A = \frac{\sum_{i=1}^{2} B_i - \sum_{i=3}^{5} C_ik_i}{B_i + C_ik_i} + \frac{\sum_{i=3}^{5} A_ik_i}{A_ik_i + D_i} \tag{34}$$

The analysis in Table 3 reveals that

$$\sum_{i=1}^{2} B_i = B_i + C_ik_i - k_1k_2k_3(AB - A/K_3) - k_1k_2k_4A \tag{35}$$

$$\sum_{i=3}^{5} C_ik_i = 2(B_i + C_ik_i) - k_1k_2k_3(AB - A/K_3) - k_1k_2k_4A \tag{36}$$

$$\sum_{i=3}^{5} A_ik_i = 2(A_ik_i + D_i) \tag{37}$$

Substitution of Eqs. (35), (36) and (37) in Eq. (34) shows that the sum of the friction coefficients with respect to the rate of disappearance of substrate A equals one, as well. Finally, the friction coefficients with respect to the slipping rate follow from variable selection in rate Eq. (26). For $i = 1,2,5$ the rate equation takes the form of Eq. (11) and the friction coefficients are given by Eqs. (16) and (32) and for $i = 3,4$ the rate equation takes the form of Eq. (20) and the expression for the friction coefficients is of the form of Eqs. (21) and (33). An expression analogous to Eq. (34) follows for the sum of the friction coefficient with respect to the slipping rate

$$\sum_{i=1}^{5} f_i^{slip} = \frac{\sum_{i=1}^{2,5} B_i - \sum_{i=3}^{4} C_ik_i}{B_i + C_ik_i} + \frac{\sum_{i=3}^{4} A_ik_i}{A_ik_i + D_i} \tag{38}$$

Analysis of the relevant terms in Table 3 indicates that the second term on the right hand side equals 1 and that the two summations in the first term are identical. Consequently, the sum over the friction coefficients with respect to the slipping rate equals one as well.

In summary, in a partially coupled enzyme the summation theorem applies to all the fluxes through the enzyme. In the present example

$$\sum_{i=1}^{5} f_i^B = \sum_{i=1}^{5} f_i^A = \sum_{i=1}^{5} f_i^{slip} = 1 \tag{39}$$

The numerical values for the friction coefficients of one transition may be the same with respect to all rates, i.e., f_1 and f_2, or different. Furthermore, the sum of a subset of friction coefficients may exceed the value of 1 and, conse-

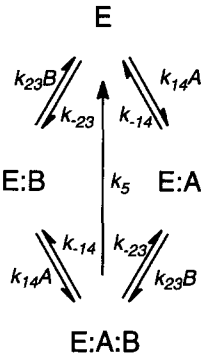
quently, transitions with negative friction coefficients exist.

4. The group rule

The friction coefficient of a transition measures the effect on the rate of a infinitesimal small change in the activation energy of that transition while all other activation energies are kept constant (Eq. 7). In the case of large kinetic schemes, characterized by many states and transitions, it is not convenient to deal with each rate constant individually. Instead, several similar transitions are grouped together and a rate constant is assigned to the group [8]. The rate equation will contain just this rate constant and consequently, according to Eq. (7), all transitions in the group will have the same friction coefficient. The friction coefficient of a group measures the effect on the rate of an infinitesimal small change in the activation energy of all the transitions in the group at the same time. The summation theorem still applies but the summation is over the groups instead of over the transitions. The group rule relates the friction coefficients of groups and transitions. It states that the friction coefficient of a group ($f_{ijk\_}$) equals the sum of the friction coefficients of the transitions (f_i, f_j, f_k,..) belonging to that group

$$f_{ijk\ldots} = f_i + f_j + f_k + \ldots \tag{40}$$

Rate constants k_1, k_{-1}, k_4 and k_{-4} in scheme IV describe the binding of substrate A to the enzyme. By assigning different rate constants to the two binding equilibria the possibility is left open that the A and B binding sites interact cooperatively. If this possibility is not considered the two transitions may be treated as one group with rate constants k_{14} and k_{-14}. Then, the two binding equilibria for substrate B form a group as well, with rate constants k_{23} and k_{-23} (Scheme VI).



Scheme VI

The rate equation follows from equating k_1 and k_4 to k_{14} and k_2 and k_3 to k_{23} in rate Eq. (20). Table 3 shows the resulting terms in the numerator and denominator. It follows for the rate equation as a function of the rate constants

$$v(k_i) = \frac{P_i k_i + Q_i k_i^2}{R_i + S_i k_i + T_i k_i^2} = \frac{\eta}{\Delta} \tag{41}$$

Note that η and Δ in Eqs. (20) and (41) are the same and that the corresponding terms in Tables 2 and 4 are the same. However, subscript i in Eq. (41) ranges from 1 to 3, one for the group of the substrate A binding equilibria (k_{14}), one for the group of the substrate B binding equilibria (k_{23}) and the product forming transition (k_5). From the definition of the friction coefficient (Eq. 7) it follows

$$f_i = \frac{P_i k_i + 2Q_i k_i^2}{\eta} - \frac{S_i k_i + 2T_i k_i^2}{\Delta} \tag{42}$$

and consequently

$$\sum_{i=1}^{3} f_i = \frac{\sum_{i=1}^{3} P_i k_i + 2\sum_{i=1}^{3} Q_i k_i^2}{\eta} - \frac{\sum_{i=1}^{3} S_i k_i + 2\sum_{i=1}^{3} T_i k_i^2}{\Delta} \tag{43}$$

Table 4
Analysis of numerator η (top) and denominator Δ (bottom) in rate Eq. (41)

η	$P_{14}k_{14}$	$P_{23}k_{23}$	P_5k_5		
$k_{14}k_{23}^2k_5\,(AB/K_2 + AB^2)$	*		*		
$k_{14}^2k_{23}k_5\,(AB/K_1 + A^2B)$		*	*		
Δ	$S_{14}k_{14}$	$S_{23}k_{23}$	S_5k_5	$T_{14}k_{14}^2$	$T_{23}k_{23}^2$
$k_{14}k_{23}^2\,(1/K_1K_2K_3 + A/K_2K_3 + B/K_3 + AB/K_2 + B/K_2K_4 + AB/K_3 + B^2/K_4 + AB^2)$	*				*
$k_{14}^2k_{23}\,(1/K_1K_2K_4 + A/K_2K_4 + B/K_1K_4 + AB/K_1 + A/K_1K_3 + AB/K_4 + A^2/K_3 + A^2B)$		*		*	
$k_{14}k_{23}k_5\,(1/K_1K_2K_5 + A/K_2 + B/K_1 + AB)$	*	*	*		
$k_{14}^2k_5\,(A/K_1 + A^2)$			*	*	
$k_{23}^2k_5\,(B/K_2 + B^2)$			*		*

Numerator η and denominator Δ are the sum of the terms in the first column. P_ik_i (top) and S_ik_i and $T_ik_i^2$ (bottom) equal the sum of the terms indicated by the asterisk (*) in the corresponding column. The remaining terms equal the $Q_ik_i^2$ – terms (top) and R_i-terms (bottom). Each term in η contributes two times to the respective P_ik_i-terms and one time to the Q_i-terms. Therefore, it follows that $\Sigma P_ik_i = 2\eta$ and $\Sigma Q_i = \eta$. The bottom table shows that each term in Δ contributes three times to the sum of S_ik_i and $2T_ik_i^2$. It follows that $\Sigma S_ik_i + 2\Sigma T_ik_i^2 = 3\Delta$.

The analysis of the contribution of the terms in the numerator and denominator Table 4 shows that

$$\sum_{i=1}^{3} P_i k_i + 2\sum_{i=1}^{3} Q_i k_i^2 = 4\eta \sum_{i=1}^{3} S_i k_i + 2\sum_{i=1}^{3} T_i k_i^2 = 3\Delta \tag{44}$$

demonstrating that the summation over the friction coefficients of the groups indeed adds up to unity. Comparison of Table 2 and 4 shows that

$$A_1 k_1 + A_2 k_2 = P_{14} k_{14} + 2Q_{14} k_{14}^2$$
$$C_1 k_1 + C_2 k_2 = S_{14} k_{14} + 2T_{14} k_{14}^2 \tag{45}$$

The group rule follows from Eqs. (21), (42) and (45)

$$f_{14} = \frac{P_{14} k_{14} + 2Q_{14} k_{14}^2}{\eta} - \frac{S_{14} k_{14} + 2T_{14} k_{14}^2}{\Delta}$$
$$= \frac{A_1 k_1}{\eta} - \frac{C_1 k_1}{\Delta} + \frac{A_4 k_4}{\eta} - \frac{C_4 k_4}{\Delta} \tag{46}$$

or

$$f_{14} = f_1 + f_4 \tag{47}$$

It should be stressed that the friction coefficient of transitions in a group (e.g., f_{14}) are only identical when they are treated as a group. In general, when treated individually, they will not be the same (e.g., $f_1 <> f_4$).

5. Implementation of the friction coefficient in a numerical analysis

The friction coefficient measures the effect of a infinitesimally small change in the friction of one transition or a group of transitions on the turnover rate (Eq. 7). The technique to compute the friction coefficient in the framework of a numerical analysis of kinetic schemes for which no analytical rate equation is available is to change the back- and forward rate constants of a particular transition (k^+ and k^-, respectively) by a small but finite fraction (Δ) and recalculate the turnover rate (v) [8]. Then, the friction coefficient equals

$$f_i = \frac{v(k_i^+, k_i^-) - v(k_i^+ + \Delta k_i^+, k_i^- + \Delta k_i^-)}{v(k_i^+, k_i^-)\,\Delta} \tag{48}$$

The summation theorem provides a test for the error introduced by the finite change in the rate constants. The friction coefficients of the 12 groups of rate constants in the proposed kinetic scheme for the mannitol permease of *Escherichia coli* [8] were calculated according to equation 48 with different values for Δ. The sum of these friction coefficients (Σ) was smaller than 1. Fig. 2 reveals that the deviation from 1 increases linear with Δ. Back-extrapolation to $\Delta = 0$, shows that the summation theorem is valid for this complex mechanism (36 states, 168 transitions). A value for Δ of 0.5–1% resulting in Σ values of 0.9975–0.995 seems to be appropriate for most purposes. Errors introduced by round-off errors of the computer are much smaller than the error introduced by the finite value of Δ. Using the Pascal single or double format for real numbers results in differences in Σ in the fifth decimal. It should be noted that these calculations are scheme- and condition-dependent and larger errors may occur.

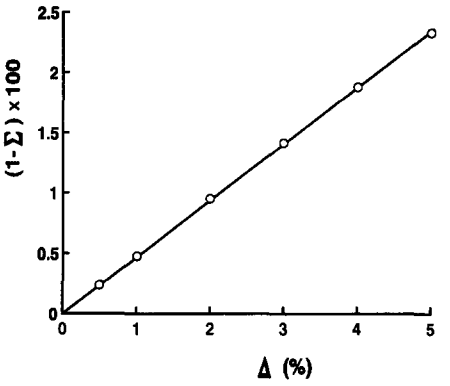


Fig. 2. The sum of the friction coefficients (Σ) as a function of the relative increase in the friction (Δ) in a numerical analysis. The kinetic scheme used for this calculation describes dimeric enzyme II^{mtl} of the phospho*enol*pyruvate-dependent phosphotransferase of *Escherichia coli* [8]. The reaction catalysed is mannitol phosphorylation by P-HPr, a small protein substrate. The scheme involves 36 states and 168 transitions that are subdivided in 12 groups. The forward and backward rate constants pertinent to these groups in the calculations were listed in the 'restricted set' [8]. The friction coefficients of the groups were calculated with different relative increases in the forward and backward rate constants (Δ) by using the computer program CACES [8] and summed to give SUM. The concentrations of mannitol and P-HPr were 1 μM and 3 μM, respectively. The product concentrations were zero.

6. Relation to other kinetic parameters

The friction coefficient is not a measurable parameter. It is a tool in the analysis of the kinetic behaviour of complex kinetic schemes. It is defined for each transition in the scheme and, thus, shows what goes on inside the kinetic scheme. By sensing the dominant rate determining transitions in the scheme, analysis of the friction coefficients shows why the enzyme behaves as it does and, moreover, provides insight in the principles of enzyme kinetics. The analytical power of the friction coefficient will be demonstrated by analysing the friction in the substrate binding steps in the single substrate reaction shown in Scheme I and the consecutive reaction shown in Scheme III.

6.1. Friction in the substrate binding steps

Combination of Eqs. (11) and (16) and substituting the value of $A_i k_i$ for the single substrate reaction shown in

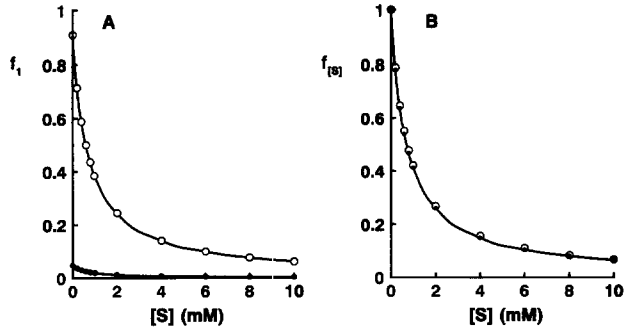


Fig. 3. (A) The friction coefficient of the substrate binding step in Scheme I at different substrate concentrations for a very efficient enzyme (○) and an enzyme that approaches Michaelis–Menten type of kinetics (●). The rate constants used for the calculation were as depicted in the schemes below for (○) and (●), respectively.

$$S+E \underset{10}{\overset{50}{\rightleftharpoons}} E{:}S \underset{100}{\overset{100}{\rightleftharpoons}} E{:}P \xrightarrow{100} E+P$$

$$S+E \underset{100}{\overset{100}{\rightleftharpoons}} E{:}S \underset{10}{\overset{10}{\rightleftharpoons}} E{:}P \xrightarrow{10} E+P$$

With the time constants in s^{-1} and the concentrations in mM, the K_m values are 0.73 (○) and 0.7 (●) and the turnover numbers, 33.3 (○) and 3.33 (●). (B) The control over the rate exerted by the substrate concentration in both schemes.

Scheme I results in the following expression for the friction coefficient of the association/dissociation step of substrate S

$$f_1 = v/k_1 S \tag{49}$$

Eq. (49) provides an alternative definition for the friction coefficient in the case of substrate binding steps: the ratio of the true rate and the maximal attainable rate when substrate association would be the only rate determining step. In case the true rate would equal the maximal attainable rate the friction coefficient becomes 1 which is in line with the definition. The relation between the friction coefficient and the kinetic parameters K_m and k_{cat} that are normally used to characterize the enzymatic reaction follows from substitution of the rate equation in terms of K_m and k_{cat}:

$$f_1 = \frac{k_{cat}}{k_1}\frac{1}{K_m + S} \tag{50}$$

The friction coefficient approaches zero as substrate concentration S increases (Fig. 3); the substrate binding step is not rate determining under V_{max} conditions. At very low substrate concentrations, f_1 reaches a maximum value equal to

$$f_1^{max} = \frac{k_{cat}/K_m}{k_1} = \frac{1}{1 + \frac{k_{-1}}{K_2 k_3} + \frac{k_{-1}}{k_2}} \tag{51}$$

which is smaller than 1. Combining Eqs. (50) and (51) shows that the friction coefficient is half the maximal friction coefficient when $S = K_m$. More importantly, the analysis shows that the substrate binding step does not become fully rate determining at zero substrate concentrations. The maximal friction approaches 1 when the second order rate constant of the enzymatic reaction (k_{cat}/K_m, associated with the catalytic efficiency) becomes equal to the substrate association constant (k_1). The right-hand side of Eq. (51) shows that this condition is favoured when the substrate dissociation rate constant (k_{-1}) is small compared to the forward rate constants. In Michaelis–Menten type of kinetics the dynamics of the substrate binding equilibrium is much higher than the steps that lead to product formation (i.e., $k_{-1} \gg k_2$). Then, the friction coefficient of the substrate binding step is small over the whole substrate concentration domain, also below the K_m for the substrate where the rate is determined by the substrate concentration (see Fig. 3A).

The control over the rate of the substrate association/dissociation step (the friction coefficient) is not the same as the control over the rate exerted by the substrate concentration. Assuming that the energy profile in Fig. 1 represents a substrate binding step with state E_j being the bound state, the friction coefficient measures the effects of a change in energy level $G^{\#}$, whereas the concentration of the substrate effects energy level G_i. By analogy, the control of the rate by the substrate concentration ($f_{[S]}$) equals

$$f_{[S]} = \frac{[S]}{v}\frac{\delta v}{\delta [S]} = \frac{K_m}{K_m + [S]} \tag{52}$$

The right-hand side of Eq. (52) gives the solution for

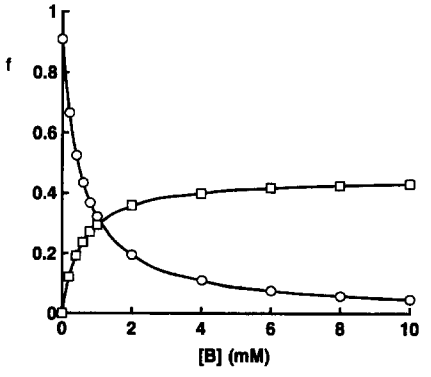


Fig. 4. Distribution of the friction over the substrate binding steps in a 'ping-pong' mechanism (Scheme III). Shown are the friction coefficients for the A (□) and B (○) binding steps at increasing concentrations of substrate B. The rate constant used for the calculations were as follows:

$$A+E \underset{10}{\overset{50}{\rightleftharpoons}} E{:}A \xrightarrow{100} {}^{*}E$$

$$B+{}^{*}E \underset{10}{\overset{50}{\rightleftharpoons}} E{:}B \xrightarrow{100} E+P$$

With the time constants in s^{-1} and the concentrations in mM, k_{cat} would be 50 s^{-1} and $K_m^A = K_m^B = 1.1$ mM.

any rate equation that describes a simple saturation curve characterized by a V_{max} and K_m. Clearly, the control over the rate by the substrate concentration is a value ranging from 1 at zero substrate concentration to zero at infinite substrate concentration, independent of the dynamics of the binding equilibrium (Fig. 3B).

6.2. Distribution of friction over the kinetic scheme

The expressions for the friction coefficients in the A and B binding steps in the kinetic mechanism depicted in Scheme III are analogues to Eq. (49):

$$f_1 = \frac{v}{k_1 A} \quad f_3 = \frac{v}{k_3 B} \tag{53}$$

The friction coefficient of substrate A (B) decreases from a maximal value that is smaller than 1 at zero A (B) concentration to zero at infinite A (B) concentration. The summation theorem requires that a decreased friction in the B binding step (Fig. 4) is compensated for by an increase in the friction in the other steps, one of which is the binding step of substrate A. Consequently, the rate control of the binding step of substrate A increases with an increasing concentration of substrate B and a higher concentration of substrate A will be necessary to make the rate independent of A. The redistribution of the control over the scheme after a change in the conditions explains why in the consecutive mechanism the apparent affinity constant for one substrate depends on the concentration of the other substrate. The extent to which a decreased friction in the binding step of substrate B leads to an increase in the friction in the binding step of substrate A depends on the concentration of A.

The example demonstrates two important points: (i) the distribution of friction over the different steps depends on the steady state condition of the enzyme, and (ii) a change in the friction in one step is always accompanied by changes in the frictions in other steps.

References

[1] Fell, D.A. (1992) Biochem. J. 286, 313–330.
[2] Schuster, S. and Heinrich, R. (1992) Biosystems 27, 1–15.
[3] Westerhoff, H.V. and Van Dam, K. (1987) Thermodynamics and Control of Biological Free Energy Transduction, Elsevier, Amsterdam.
[4] Jensen, P.R., Westerhoff, H.V. and Michelson, O. (1993) Eur. J. Biochem. 211, 181–191.
[5] Groen, A.K., van der Meer, R., Westerhof, H.V., Wanders, R.J.A., Akerboom, R.J.M. and Tager, J.M. (1982) In: Metabolic compartmentation (Sies, H., ed.), pp. 9–37, Academic Press, New York.
[6] Brown, G.C. and Cooper, C.E. (1993) Biochem. J. 294, 87–94.
[7] Kholodenko, B.N. and Westerhoff, H.V. (1994) Biochim. Biophys. Acta 1208, 294–305.
[8] Lolkema, J.S. (1993) J. Biol. Chem. 268, 17850–17860.
[9] Ray, W.J. (1983) Biochemistry 22, 4625–4637.
[10] Pütner, I.B., Sarkar, H.K., Poonian, M.S. and Kaback, H.R. (1986) Biochemistry 25, 4483–4485.
[11] Matzke, E.A., Stephenson, L.J. and Brooker, R.J. (1992) J. Biol. Chem. 267, 19095–19100.
[12] Knol, J., Lolkema, J.S. and Poolman, B. (1995) J. Biol. Chem. 270, 12995–13003.
[13] Yamaguchi, A., Adachi, K., Akasaka, T., Ono, N. and Sawai, T. (1991) J. Biol. Chem. 266, 6045–6051.
[14] Lolkema, J.S. and Poolman, B. (1995) J. Biol. Chem. 270, 12670–12676.
[15] Brooker, R.J. (1991) J. Biol. Chem. 266, 4131–4138.
[16] Walz, D. and Caplan, S.R. (1988) Cell Biophys. 12, 13–28.
[17] King, E.L. and Altman, C. (1956) J. Phys. Chem. 60, 1375.
[18] Dixon, M. and Webb, E.C. (1979) in Enzymes, 3rd Ed., Longman, London.
[19] Marcus, R.A. (1969) J. Am. Chem. Soc. 91, 7224–7225.